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硕士学位论文

海马齿生态浮床植物根际脱氮过程  
及影响因素的研究

Study on the nitrogen removal process and influencing  
factors in the rhizosphere of *Sesuvium portulacastrum*  
floating bed

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## 摘要

大气是地球氮元素储量最大的氮库，由于氮气的三键结构十分稳定，打开它所需的能量较高，因此在工业革命以前，主要靠闪电固氮和生物固氮将其转化为人和生物可以利用的含氮化合物。然而随着人类社会的不断发展，为了满足人们生产生活的需要，大量氮元素被通过工业的方式固定下来，并随着化肥的滥用积累在环境中，造成氮循环的破坏，并产生水体富营养化现象。富营养化带来的恶果就是水体藻类大量繁殖，溶解氧急剧降低，造成鱼虾等水生生物的死亡等。因此，想要根除富营养化现象就必须将水体过量的氮、磷营养盐从水体脱除。目前人们所发现的自然界的生物脱氮过程有两个，反硝化过程和厌氧氨氧化(Anammox)过程。在反硝化过程中，硝酸盐经过异化硝酸盐还原作用被逐步还原为一氧化氮、氧化亚氮和氮气，在厌氧氨氧化过程中，铵盐和亚硝酸盐或硝酸盐发生反应，生成氮气。这两种过程需要在反硝化细菌和厌氧氨氧化细菌的参与下才能完成。

近年来，人们开发出许多技术来治理富营养化水体，如人工湿地技术、生物操纵技术、生态浮床技术等，并且这些技术已经成功应用于富营养化水体的治理中。其中生态浮床技术由于其无可比拟的优势，成为了极具发展潜力的生态修复技术。然而，生态浮床技术却一直难以应用于富营养化问题同样严重的海水中，因为难以找到一种耐盐的浮床植物。本实验室经过多年研究，筛选出海马齿、盐角草等耐盐植物作为浮床植物，并已成功应用于富营养化海水的治理中。

在实验室之前的研究基础上，本研究通过构建模拟海马齿生态浮床，设置不同的溶解氧、盐度和海马齿根系长度梯度，利用实时荧光定量 PCR 技术，对水体中的 *nosZ* 基因和 *hzo* 基因进行定量，研究溶解氧、盐度和海马齿根系长度对浮床植物根际微生物的影响，并在最佳溶解氧、盐度和海马齿根系长度的条件下对海马齿生态浮床系统的脱氮途径和脱氮量进行研究，研究主要结论如下：

1、在低溶解氧（ $DO < 0.6 \text{ mg/L}$ ）条件下，海马齿生态浮床系统植物根际水体中 *hzo* 基因丰度为  $67.13 \text{ copies/mL}$ ，*nosZ* 基因的丰度为  $1927.13 \text{ copies/mL}$ ，高于中溶解氧（ $DO \sim 2.0 \text{ mg/L}$ ）和高溶解氧（ $DO > 7.0 \text{ mg/L}$ ）条件下，因此我们认为低溶解氧条件更有利于反硝化细菌和厌氧氨氧化细菌的生长。此外，在所有溶解氧条件下，反硝化细菌在总脱氮细菌中数量占比均大于 94%，相应的厌氧氨氧

化细菌在总脱氮细菌中的数量占比均小于 6%，因此我们认为海马齿生态浮床根际脱氮微生物群落中反硝化过程占据主导作用。

2、在低溶解氧 ( $DO < 0.6 \text{ mg/L}$ )、高盐度 ( $Sal \sim 30$ ) 条件下，海马齿生态浮床系统植物根际水体中 *hzo* 基因丰度为  $79.02 \text{ copies/mL}$ ，*nosZ* 基因的丰度为  $3093.31 \text{ copies/mL}$ ，总脱氮基因拷贝数高于中盐度 ( $Sal \sim 20$ ) 和低盐度 ( $Sal \sim 0.5$ ) 条件下，因此我们认为高盐度条件更有利于反硝化细菌和厌氧氨氧化细菌的生长，这种现象可能与体系中微生物的来源有关。在所有盐度条件下，反硝化细菌在总脱氮细菌中的数量占比均大于 92%，相应的厌氧氨氧化细菌在总脱氮细菌中的数量占比均小于 8%，因此我们认为海马齿生态浮床根际脱氮微生物群落中反硝化过程占据主导作用。

3、在低溶解氧 ( $DO < 0.6 \text{ mg/L}$ )、高盐度 ( $Sal \sim 30$ ) 条件下，海马齿生态浮床系统植物根际水体中的 *hzo* 基因拷贝数不随根长的改变而改变，而 *nosZ* 基因拷贝数则与根长呈显著的负相关。我们推测这是因为在全根（根冠比为 0.09）条件下，异养的反硝化细菌需要植物根系分泌物作为碳源供应生长，因此附着在根系表面，因而根际水体中丰度比半根条件和无根条件降低；而厌氧氨氧化细菌属化能自养型细菌，有机物对其有抑制作用，因此其较喜欢生活在根际水体中而非根系表面，因此会出现全根、半根和无根条件下其丰度在 0.05 的显著性水平上无显著差异。

4、在低溶解氧 ( $DO < 0.6 \text{ mg/L}$ )、高盐度 ( $Sal \sim 30$ ) 条件下，通过对海马齿生态浮床系统植物根际脱氮过程的研究，发现海马齿生态浮床对与水体氮营养盐具有较强的净化能力，在培养 7 天时，其对水体氮元素的平均脱除速率可达到  $1.36 \text{ mg/d}$ 。此外，海马齿生态浮床系统根际脱氮过程存在植物根系吸收以及根际微生物脱氮两种作用，实验前期根际微生物脱氮作用占据主导，而实验后期则是植物根系吸收作用占据主导。

**关键词：**生态浮床；海马齿；根际微生物；脱氮过程；影响因素

## Abstract

Atmosphere is the biggest nitrogen pool on the earth. Before the industrial revolution, nitrogen gas transformed to nitrogen-containing compound that can be used by human beings mainly via lightning and biological nitrogen fixation cause the high energy needed to break the very stable three key structure. But with the developing of the human society, lots of nitrogen element was fixed in industrial ways in order to reach the needs of production and life, and it accumulates in the environment with the abuse of chemical fertilizer causing destruction of nitrogen cycle and producing the phenomenon of eutrophication. Eutrophication can lead to algae blooms in water, sharp reduction of dissolved oxygen, and the death of aquatic organism like fish and shrimps. So excess nitrogen and phosphorus nutrients must be removal from water in order to eliminate eutrophication. Nowadays people find to biological nitrogen process in nature: denitrification and anaerobic ammonia oxidation (anammox). In the process of denitrification, nitrate is gradually reduced into nitric oxide, nitrous oxide and nitrogen gas by dissimilatory nitrate reduction. In the process of anammox, reaction of ammonium and nitrite or nitrate was occurred, and generate the nitrogen gas. These two processes must be completed in the present of denitrifying bacteria and anammox bacteria.

In recent years, people have developed many technologies to control eutrophication, such as artificial wetlands technology, biomanipulation technology and biological floating bed technology, and these technologies have been successfully used in the govern of eutrophication water, among which biological floating bed technology has become the most development potential ecological restoration technology for its incomparable advantage. However, biological floating bed technology can still not be used in the eutrophication seawater for hardly finding a haloduric floating bed plant. After study for so many years, *Sesuvium portulacastrum* and *Salicornia europaea* were screened out for floating bed plant, and have been already used in governing the eutrophication seawater.

Based on previous studies in our laboratory, we build simulative *Sesuvium portulacastrum* ecological floating bed, and set different DO, salinity and root length gradient to quantify *nosZ* gene and *hzo* gene in the water by using real-time quantify PCR technique, in order to find how DO, salinity and root length influence rhizosphere microorganisms of floating bed. And we study nitrogen removal pathways and amount under the best DO, salinity and root length conditions. Conclusions are as follows:

1. Under the low DO condition ( $\text{DO} < 0.6 \text{ mg/L}$ ), the abundance of *hzo* gene is 67.13 copies/mL in the rhizosphere water of the *Sesuvium portulacastrum* ecological floating bed system, while the abundance of *nosZ* gene is 1927.13 copies/mL, which is higher than that in the condition of middle DO ( $\text{DO} \sim 2.0 \text{ mg/L}$ ) and high DO ( $\text{DO} \sim 7.0 \text{ mg/L}$ ). So we think that low DO condition is propitious to denitrifying bacteria and anammox bacteria. What's more, denitrifying bacteria abundance accounts for more than 94% in total nitrogen removal bacteria under all DO condition, and anammox bacteria abundance accounts for less than 6% in total nitrogen removal bacteria correspondingly. So we think denitrification process plays the leading role in the rhizosphere microbial community of the *Sesuvium portulacastrum* ecological floating bed.

2. Under the low DO ( $\text{DO} < 0.6 \text{ mg/L}$ ) and high salinity ( $\text{Sal} \sim 30$ ) condition, the abundance of *hzo* gene is 79.02 copies/mL in the rhizosphere water of the *Sesuvium portulacastrum* ecological floating bed system, while the abundance of *nosZ* gene is 3093.31 copies/mL, which is higher than that in the condition of middle salinity ( $\text{Sal} \sim 20$ ) and low salinity ( $\text{Sal} \sim 0.5$ ). So we think that high salinity condition is propitious to denitrifying bacteria and anammox bacteria, and this phenomenon may related to the source of the microorganism in the system. What's more, denitrifying bacteria abundance accounts for more than 92% in total nitrogen removal bacteria under all salinity condition, and anammox bacteria abundance accounts for less than 8% in total nitrogen removal bacteria correspondingly. So we think denitrification process plays the leading role in the rhizosphere microbial community of the *Sesuvium portulacastrum* ecological floating bed.

3. Under the low DO ( $\text{DO} < 0.6 \text{ mg/L}$ ) and high salinity ( $\text{Sal} \sim 30$ ) condition, the abundance of *hzo* gene does not change with the change of root length in the rhizosphere water of the *Sesuvium portulacastrum* ecological floating bed system, but the abundance of *nosZ* gene and root length are significantly negative correlated. We speculate that heterotrophic denitrifying bacteria need plant rhizosphere discharge to be the carbon source to live under the whole root condition (root shoot ratio=0.09), therefore they like to adhere to the surface of the plant roots. So the abundance in the rhizosphere water is lower than that under half root and non root conditions; However, anammox bacteria belong to chemolithoautotrophy bacteria, and organic matter have inhibitory effect on them, so that they like to live in the rhizosphere water rather than on the surface of the plant roots. So the abundance has no significant correlation under all root length condition.

4. Under the low DO ( $\text{DO} < 0.6 \text{ mg/L}$ ) and high salinity ( $\text{Sal} \sim 30$ ) condition, we find that *Sesuvium portulacastrum* ecological floating bed has fairly strong ability to remove nitrogen nutrient in the water by studying the rhizosphere nitrogen removal process of the *Sesuvium portulacastrum* ecological floating bed system. On the 7th day, nitrogen removal rate can reach  $1.36 \text{ mg/d}$ . What's more, there exist absorption of roots and nitrogen removal by rhizosphere microorganisms in the rhizosphere of *Sesuvium portulacastrum* ecological floating bed, and the latter plays the leading role at the early time of the experiment and the former plays the leading role at the later.

**Key words:** Ecological floating bed; *Sesuvium portulacastrum*; Rhizospheric microorganisms; Nitrogen removal process; Influencing factors

## 第一章 绪论

### 1.1 生物脱氮

氮(N)作为太阳系中总量排在第五的元素,是核酸和蛋白质合成的必不可少的元素,已被人们视为在其它星球发现生物的潜在最佳生物标志元素<sup>[1]</sup>。事实上,生物对N的需求量是非常大的。根据不同的生命体构成,进入细胞的每100个碳原子中就伴随着2-20个氮原子<sup>[2]</sup>。然而,能被生物直接利用的氮相比于整个氮库来说是非常少的。生物圈中最大的氮库是大气,氮以氮气(N<sub>2</sub>)的形式存在。在海平面上,氮气约占大气体积的79%。但由于氮气的三键结构十分稳定,打开三键需要的能量非常高,因此除了少数固氮微生物,其它生物很难直接利用。所以氮元素也成为了陆地和海洋生态系统中限制初级生产力的主要因子。

氮素的存在形式有很多种,主要的存在形式按照其化合价从低到高依次为: NH<sub>3</sub> (-III)、N<sub>2</sub> (0)、N<sub>2</sub>O (+I)、NO (+II)、NO<sub>2</sub><sup>-</sup> (+III)、NO<sub>2</sub> (+IV)和NO<sub>3</sub><sup>-</sup> (+V)等。生物作用可以使氮素在这些价态之间相互转变,加之其在不同氮库的迁移,就构成了氮循环(Nitrogen cycle)。虽然人们发现氮循环中的某些现象已经有一个多世纪的历史,但直到1934年,Bass-Becking才首次提出氮循环的基本概念<sup>[3]</sup>。传统意义上的氮循环包括四个过程:固氮作用(Nitrogen fixation)、氨化作用(Ammonification)、硝化作用(Nitrification)和反硝化作用(Denitrification)。

1、固氮作用:将分子态N<sub>2</sub>转化为氨的过程叫做固氮过程。目前的固氮过程主要有人工固氮、高能固氮和生物固氮三种类型,其中生物固定的氮量远非人工固氮和高能固氮所企及,可以说自然界的固氮主要靠生物固氮,因此生物固氮在自然界氮循环中具有十分重要的意义。

2、氨化作用:氨化作用是指生物遗骸等有机含氮大分子在微生物的作用下分解,释放出氨的过程,也被称为矿化作用。氨化作用所释放的氨,一部分积累于环境中,另一部分被其他生物重新利用。氨化作用使氮从有机态转化为无机态,是实现氮素循环的重要环节。

3、硝化作用:将氨氧化为亚硝酸盐和硝酸盐的生物反应,称为硝化作用。其中由自养型微生物引发的硝化作用称为自养型硝化作用(Autotrophic



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